American Journal of Hematology 52:108–113 (1996)

First Report of a B Cell Lymphoproliferative Disorder Arising in a Patient Treated With Immune Suppressants for Severe Aplastic Anemia

Vicki Dorr, Gary Doolittle, and Janet Woodroof

Division of Oncology, Department of Internal Medicine (V.D., G.D.), and Department of Pathology (J.W.), University of Kansas Medical Center, Kansas City, Kansas

Aplastic anemia is a disorder characterized by pancytopenia and bone marrow hypocellularity. There is some evidence that aplastic anemia may be due to suppression of hematopoiesis by activated T-suppressor cells. Thus, immunosuppressive agents have been used as an alternative to bone marrow transplantation for treatment. We report on a unique case of a patient with aplastic anemia who was treated with a course of immunosuppression including cyclosporine (CSA), anti-thymocyte globulin (ATG), and prednisone. Five months after this treatment, the patient developed a B cell lymphoproliferative disorder which was successfully treated with radiation therapy. Prior reports of CSA-associated lymphoproliferative disorders have appeared in the literature as potential side effects of immunosuppression following transplantation. This is the first report of a lymphoproliferative disorder associated with immunosuppressive treatment of aplastic anemia in a non-transplant setting. Thus, when presenting options for treatment of aplastic anemia, lymphoproliferative disorders should be included as a rare complication of immunosuppressive therapy. © 1996 Wiley-Liss, Inc.

Key words: aplastic anemia, cyclosporine A, immunosuppressive agents, lymphoproliferative disorders

INTRODUCTION

Aplastic anemia is a heterogeneous disorder of bone marrow stem cells characterized by pancytopenia and bone marrow hypocellularity. The etiology of this condition is unknown in over 50% of cases. In others, it may be secondary to myelotoxic drugs or chemicals, infection, radiation, immune disorders, or inherited disorders. The pathophysiology of bone marrow failure is uncertain, although one possible explanation may be suppression of hematopoiesis by activated T-suppressor cells [1]. As a result, immunoregulatory treatment has been implemented in an attempt to treat patients with this disorder.

Less than 20 years ago survival for patients with aplastic anemia approached only 25% [2]. Dramatic improvements have since been documented, using bone marrow transplantation with survival approaching 80% [2]. Unfortunately, not all patients are candidates for a transplant due to advanced age or lack of a human leukocyte antigen (HLA)-matched donor. Alternatively, varying combinations of immunosuppressive agents including anti-lymphocyte globulin (ALG), anti-thymocyte globulin (ATG), cyclosporine A (CSA), and prednisone have been used in the treatment of aplastic anemia.

Both ALG and ATG are thought to be similar, although not identical, in their mechanisms of action and are herein considered analogous. Initially, ALG was noted to have some effectiveness in patients treated unsuccessfully with allogeneic bone marrow transplantation for aplastic anemia. In these patients autologous hematopoietic function recovered with the use of ALG [3]. Response rates of up to 61% after ALG/ATG treatment have been reported [4]. The exact mechanism of ALG/ATG action is unknown, but it is believed to be modulated via the immunosuppressive effects of the drugs [2].

Received for publication May 2, 1995; accepted September 20, 1995.

Address reprint requests to Gary Doolittle, M.D., Division of Oncology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160-7353.

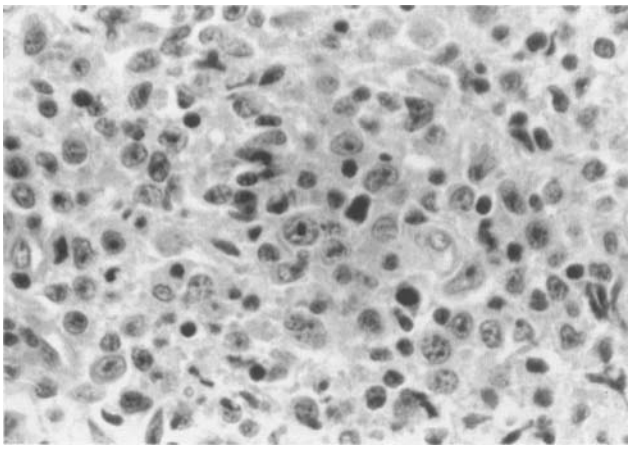

Fig. 1. Bone marrow biopsy shows 5–10% cellularity, with a decrease seen in all cell lines.

Cyclosporine, initially used as an immunosuppressive agent in organ transplantation, has now been used in many other illnesses in which an autoimmune etiology has been hypothesized. In the treatment of aplastic anemia, response rates as high as 70% have been reported [4,5]. While the exact mechanism is unknown, cyclosporine is thought to act via inhibition of the activation and proliferation of T lymphocytes. It also interrupts active immune responses, presumably by blocking the production and secretion of lymphocytic cytokines [2].

There are many reports in the literature of lymphoproliferative disorders associated with the use of CSA and/or ATG in the posttransplant setting [6–11]. We report on a unique case of a B cell lymphoproliferative disorder that developed in a patient treated with ATG and CSA for aplastic anemia.

CASE REPORT

This 17-year-old white female was first admitted in November 1989 with pancytopenia: hemoglobin (Hgb), 7.1 g/dl; white blood cell count (WBC), $0.8 \times 10^3/\mu l$, with a differential of 40% segmented cells, 6% bands, 40% lymphocytes, and 14% monocytes; and platelet count (plt), 17,000/μl. The patient had complained of easy bruising, fatigue, several episodes of epistaxis, and a heavy menstrual flow in the prior 2 weeks.

Her past medical history included herpes zoster at age 3 years (1975) and an episode of encephalitis at age 4 years (1976). She had experienced a recent non-A, non-B, non-C hepatitis 6 months prior (5/89), with associated herpes zoster (6/89). She was on no medications.

At admission, physical examination was significant for evidence of recent epistaxis and petechiae over the lower extremities. The bone marrow aspirate and biopsy demonstrated hypocellularity (5–10% cellularity) without dysplastic features, consistent with aplastic anemia (Fig. 1). Cytologic changes suggestive of parvovirus were not observed. The chemistry profile was normal. Additional laboratory studies indicated normal thyroid function, and negative titers for Epstein-Barr virus (EBV), cytomegalovirus (CMV), and Hepatitis A–C. Titers for varicella-zoster were midpositive.

Bone marrow transplantation was not feasible, as her only phenotypic match (mother) had an active CMV infection. Thus a trial of ATG, CSA, and prednisone was initiated. In January 1990, the patient received ATG (750 mg alternating with 1 g every other day for 14 days) and intravenous (IV) methylprednisolone (125 mg daily for 14 days). The patient was switched to oral prednisone and tapered off steroids. After ATG was completed, cyclosporine was begun at 250 mg orally twice daily. Serial counts are shown in Figures 2–4.

The patient did well after treatment, except for recurrent oral herpetic lesions which were treated with acyclovir. In March 1990, she reported a sore throat and her physical examination was unremarkable. She was treated with antibiotics. Her symptoms persisted, and in May the patient was noted to have a 2-cm right submental node that did not resolve with further antibiotic therapy. In June, a posterior right-sided tongue mass was noted, and a CAT scan revealed a 2-cm mass in the right tonsillar fossa obliterating the right pyriform sinus. A biopsy of this area was nondiagnostic.

The patient was admitted 2 days after the biopsy with fever, difficulty in swallowing, and shortness of breath. In the previous month, she had developed night sweats, low-grade fevers, and a 10-lb weight loss. Physical examination revealed a mass involving the right tongue base and posterior pharynx, a 2-cm right submental node, and several smaller anterior cervical lymph nodes on the right. A computerized tomography (CT) scan demonstrated rapid enlargement of the tumor to 4 cm, with partial obstruction of the airway. A tracheostomy was performed emergently, and biopsies were obtained.

Biopsy of the pharyngeal mass (Fig. 5) revealed an atypical lymphoid infiltrate with areas of necrosis. This infiltrate consisted of a polymorphic cell population composed of small, round to cleaved cells, large noncleaved cells, and immunoblasts (many with plasmacytoid features), which were shown to stain positively with CD 20 (pan-B cell marker) by paraffin immunoperoxidase. Scattered small, round lymphocytes were present, which stained positive for CD45RO (T cell marker), representing a reactive T cell component. In situ hybridization studies for EBV were negative on pariffin-embedded tissue. The lymphoid infiltrate was interpreted to represent a non-Hodgkin's lymphoma, immunoblastic type. Unfortunately, frozen tissue was not available for gene rearrangement studies, and clonality is indeterminate. A repeat bone marrow aspirate and biopsy showed a mildly hypoplastic bone marrow (20–25% cellularity) with inter-

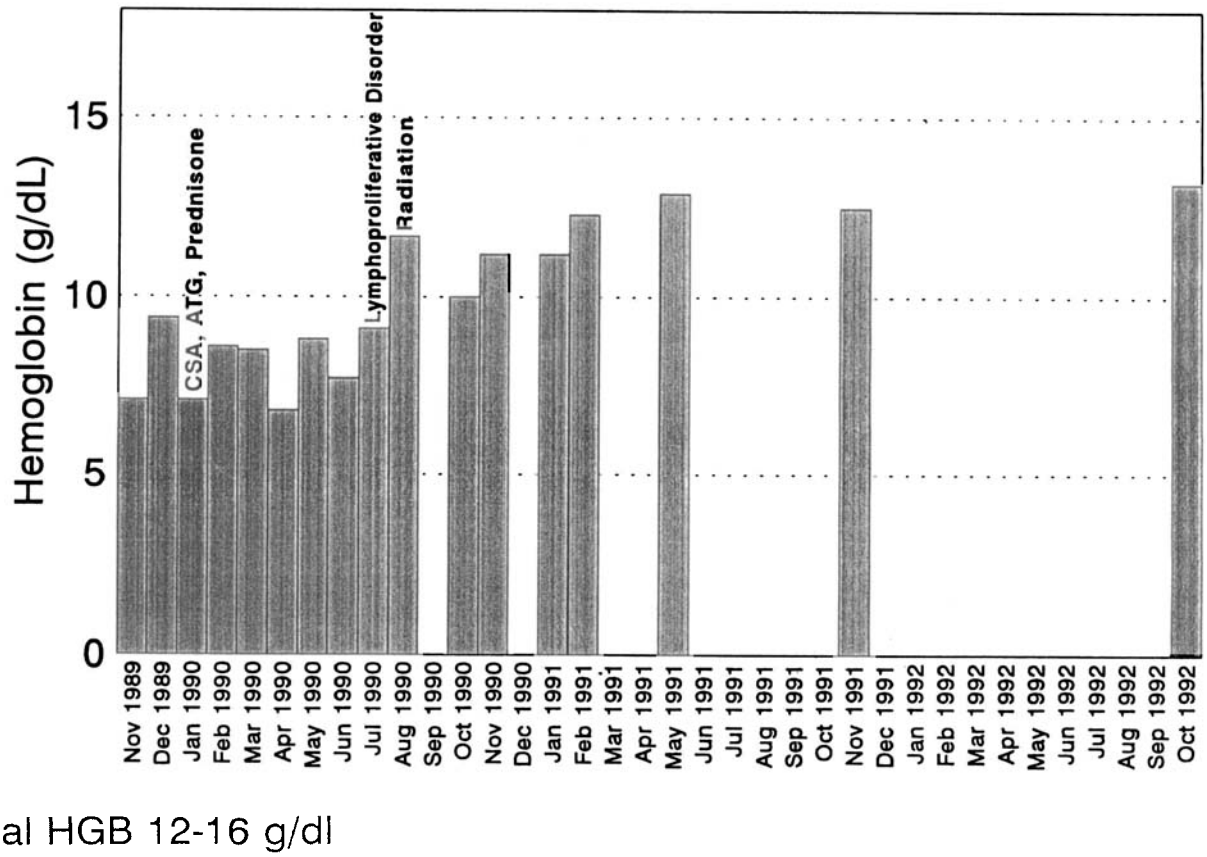

Fig. 2. Values for hemoglobin.

val increase in cellularity in all cell lines, and no evidence of lymphoma.

In light of the airway obstruction the patient was treated emergently with local radiation therapy. Chemotherapy was not used, given her recent recovery from aplastic anemia and her borderline cell counts. Her cyclosporine was discontinued. Her symptoms resolved rapidly, and repeat CAT scans demonstrated no evidence of disease. At this time, 5 years after diagnosis, the patient remains free of disease.

DISCUSSION

This report highlights a potential complication of immunosuppressive therapy used in treating aplastic anemia. We believe that the lymphoproliferative disorder resulted from the use of immunosuppressive agents, as evidenced by the recent use of CSA, ATG, and prednisone, the time course from the initiation of immunosuppressants to the onset of the lymphoproliferative disorder, the extranodal site of presentation, and the histologic appearance of the tumor. While there have been previous reports of lymphoproliferative disorders induced by immunosuppression, the vast majority of these have been in the transplant literature (heart, kidney, and bone marrow), with only rare isolated reports associated with CSA use in other conditions (psoriasis, Behcet's, and Sjogren's Syndromes [12–14]). This is the first report of a lymphoproliferative disorder occurring after immunosuppressive therapy for aplastic anemia in a nontransplant setting.

The increased risk of lymphoproliferative disorders associated with immunosuppressive therapy after transplantation has been well-documented. In a study evaluating secondary cancers after bone marrow transplantation for leukemia or aplastic anemia, a 355-fold increase in non-Hodgkin's lymphoma was found [9]. Another study cites a 1.7% detection rate of lymphoproliferative disorders with the use of a variety of immunosuppressant agents in organ transplantation [7]. All the regimens reported included CSA: prednisone and CSA were used in 21/43 cases (48.8%), and CSA, prednisone, and ALG in 3/43 cases (7.0%); in the other cases varying regimens that included CSA were used [7]. Another source reports a 28-fold increase in the relative risk of development of a lymphoproliferative disorder after CSA therapy for transplantation [15]. The time to onset of the lymphoproliferative disorder varies from 0.7–162 months after CSA use, with a median of 4.5 months and a mean of 11.7

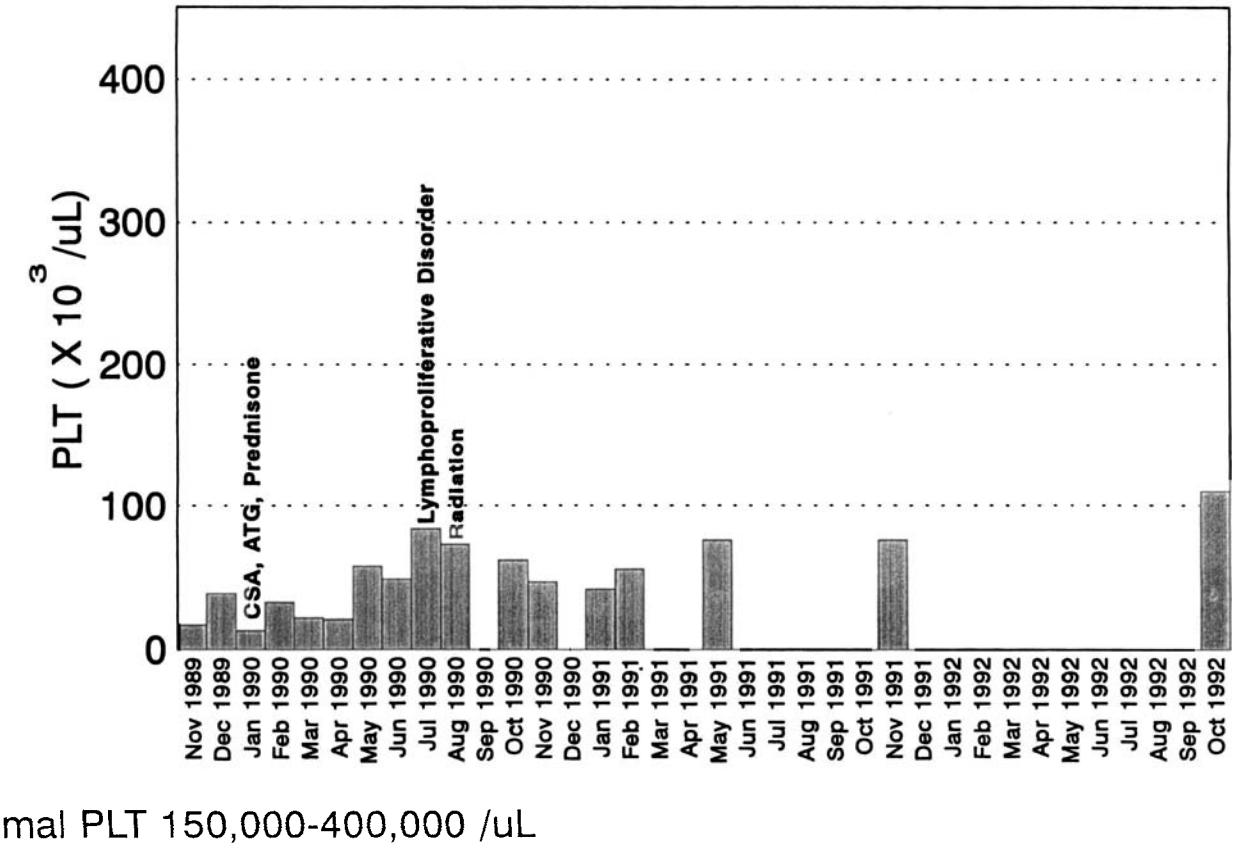

Fig. 3. Values for platelets.

months [7]. In this case the diagnosis was made 6 months after the initial immunosuppressant therapy.

Perhaps the mechanism by which CSA is able to induce a lymphoproliferative change can be explained by examining its actions. CSA is not myelosuppressive [18]; instead, it inhibits expression of antigen-induced signals from T lymphocytes that are needed for generation of an immune response [16]. Cyclosporine specifically decreases production of IL-2 [13,19] and T cell gamma-interferon [20]. Additionally, CSA inhibits T-suppressor activity, which is responsible for the control of B cell proliferation [8]. In animal studies, the administration of CSA has led to a nonmalignant B cell proliferation in the spleen and lymph nodes [16]. Epstein-Barr virus may play a role herein by inducing an intense B cell proliferation which is unchecked in the CSA-treated patient [8]. Additionally, CSA increases IL-6, which is thought to activate B cells and has been shown to support the growth of EBV-transformed B-cell cultures [21]. In fact, the majority of CSA-associated lymphoproliferative disorders are of B cell origin, which lends credence to this theory [6–9]. CSA is known to induce chromosomal abnormalities, and in one study chromosome aberrations were seen in 17/25 patients maintained on CSA for longer than 1 year [22]. Finally, CSA has been demonstrated to induce sister chromatid exchange in vitro [23].

In animal studies, CSA alone is unable to induce a malignant transformation, but appears to require an initiating event such as a viral infection or radiation [16,17]. In the literature, the majority of CSA-associated lymphoproliferative disorders appear to be associated with a viral infection, primarily EBV [6–9]. In this case, both serologic titers and in situ hybridization for EBV on biopsies were negative. Several cases have also been associated with herpes simplex virus or cytomegalovirus [7]. In this case, the patient suffered from recurring oral herpes simplex infections, and it is possible that this virus may have played a contributing role. Hence, the mechanism by which a malignant lymphoproliferative change occurs may be a combination of an early event with a concomitant insult that impairs the ability to correct for the initiating event.

While we believe that this patient's lymphoproliferative disorder was most likely induced by use of CSA, an alternative explanation should also be considered. In this case, the patient was also treated with ATG, which has also been associated with lymphoproliferative disorders. In the precyclosporine era, several reports of lymphoma

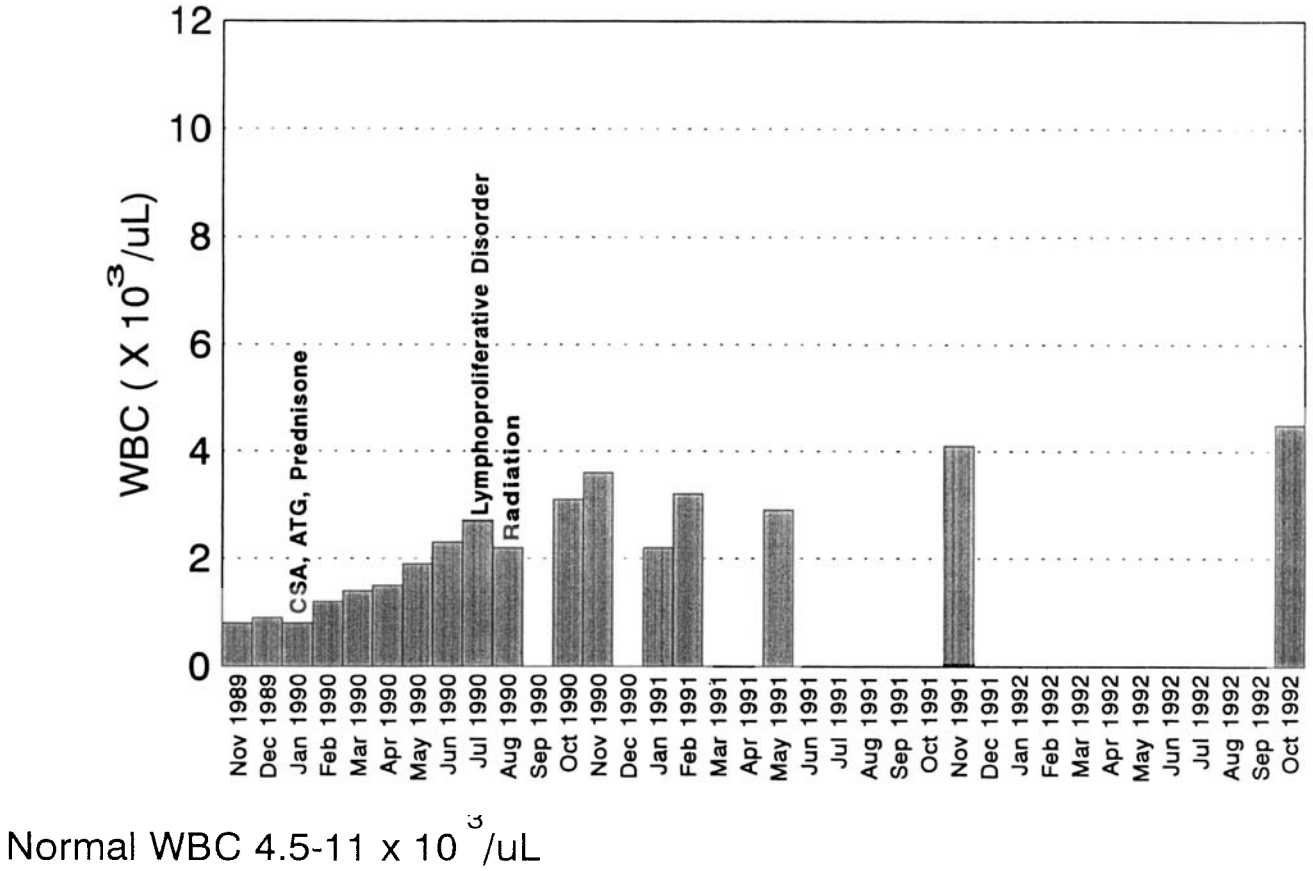

Fig. 4. Values for white blood cell count.

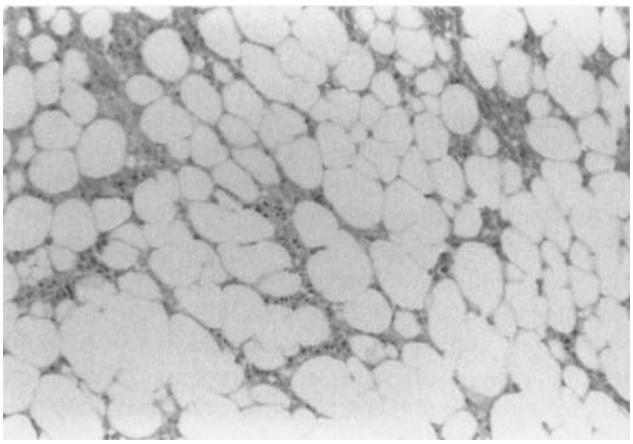

Fig. 5. Biopsy of pharyngeal mass, showing an atypical lymphoid infiltrate composed of a polymorphous B cell population with prominent immunoblasts.

associated with ATG and ALG were reported [10,24–26]. As with CSA, in these cases ATG and ALG were not given alone but in combination with other immunosuppressant agents. The actual mechanism of action of ATG and ALG is unclear, although both appear to have immunosuppressant and immunostimulatory effects. During and initially after administration, there is a decrease in the absolute peripheral lymphocyte count, with rapid recovery thereafter. ATG and ALG contain an antibody that can block the F_c receptor of IgG and limit the immune response. Both may act to improve marrow function by inducing production of hematopoietic growth factors (GM-CSF and IL-3) from lymphocytes. Additionally, ATG and ALG may directly stimulate erythroid colony growth in the marrow [2]. Unlike CSA, there is no change in the total number of T lymphocytes or in the proportion of T cell subsets. Thus, it is unclear how ATG and ALG may specifically induce a lymphoproliferative change. However, it should still be considered as a potential factor in the etiology of the lymphoproliferative disorder in this patient.

In conclusion, we believe that this represents the first reported case of a lymphoproliferative disorder occurring after immunosuppressive therapy in a patient with aplastic anemia in a nontransplant setting. The extranodal site of presentation and the histologic appearance of this lymphoma are similar to the polymorphous B cell lymphoma which is described in the classification of posttransplant lymphoproliferative disorders [27]. While

in the majority of these cases EBV was detected, in our case it was not found. It is possible that another virus played a contributing role. The most likely causative agent in this case is CSA, although the use of additional immunosuppressants (ATG and prednisone) may have been contributory. In some cases of aplastic anemia, immunosuppressant therapy may be considered before bone marrow transplantation, or may be the only option for treatment. Thus, it is important to recognize that the risk of lymphoproliferative disorders associated with immunosuppressive therapy may also exist in the nontransplant setting.

REFERENCES

1. Zoumbos NC, Gascon BP, Djeu JY, Trost SR, Young NS: Circulating activated suppressor T-lymphocytes in aplastic anemia. N Engl J Med 312:257, 1985.
2. Bjorkholm M. Aplastic anemia: Pathogenetic mechanisms and treatment with special reference to immunomodulation. J Int Med 231:575, 1992.
3. Mathe G, Amiel JL, Schwarzenberg L, Choay J, Trolard P, Schneider M, Hayat M, Schlumberger JR, Jasmin CL: Bone marrow graft in men after conditioning by antilymphocyte globulin. Br [Clin Res] Med J 2:131, 1970.
4. Bacigalupo A, Hows E, Gluckman C, Nissen C, Marsh J, Van Lint MT, Congiu M, De Planque MN, Ernst P, McCann S, Ragavashar A, Frikhofen N, Wursch A, Marmont M, Gordon-Smith EC: Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anemia (SAA): A report of the EBMT SAA Working Party. Br J Haematol 70:177, 1987.
5. Frickhofen N, Kaltwasser JP, Schrizenmeier H, Raghavachar A, Vogt HC, Herrmann F, Freund M, Neusers P, Salama A, Heimpel H: Treatment of aplastic anemia with anti-lymphocyte globulin and methylprednisolone with and without cyclosporine-A. N Engl J Med 324:1297, 1991.
6. Bernstein D, Baun D, Berry G, Dahl G, Weiss L, Stames VA, Gamberg P, Stinson EB: Neoplastic disorders after pediatric heart transplantation. Circulation 88:230, 1993.
7. Nalesnik MA, Jaffe R, Starzl TE, Demetris AJ, Porter K, Burham JA, Maowka L, Manto H, Locker J: The pathology of post-transplant lymphoproliferative disorders occurring in the setting of cyclosporine-A/prednisone immunosuppression. Am J Pathol 133:173, 1988.
8. Honda H, Barloon TJ, Franken EA, Garneau RA, Smith JL: Clinical and radiologic features of malignant neoplasms in organ transplant recipients: Cyclosporine-A treatment versus untreated patients. Am J Rad 154:271, 1990.
9. Witherspoon RP, Fisher LD, Schock G, Martin P, Sullivan KM, Sanders J, Deeg HJ, Doney K, Thomas D, Storb R, Thomas ED: Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. N Engl J Med 321:784, 1989.
10. Opely G, Henderson R: Incidence of non-Hodgkins lymphoma in kidney and heart transplant recipients. Lancet 342:1514, 1993.
11. Penn I: Cancers after cyclosporine-A treatment. Transplant Proc [Suppl] 20:276, 1988.
12. Brown LA, Campbell WM, Pringle JH, Lauder I: High grade T-cell lymphoma following treatment with cyclosporine-A. Histopathology 19:225, 1991.
13. Koo JY, Kadonaga JN, Wintroub BV, Lozada-Nur FI: The development of B-cell lymphoma in a patient with psoriasis treated with cyclosporine-A. J Am Acad Dermatol 26:836, 1992.
14. Fye KH, Daniels TE, Zulman J, Michalski JP, Jaffe R, Talal N: Aplastic anemia and lymphoma in Sjogren's syndrome. Arthritis Rheum 23:1321, 1980.
15. Cockburn ITR, Krupp P: The risk of neoplasms in patients treated with cyclosporine-A. J Autoimmunity 2:723, 1989.
16. Cohen DJ, Loertsher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB: Cyclosporine: A new immunosuppressive agent for organ transplant. Ann Int Med 101:667, 1984.
17. Hattori I, Kunz HW, Gill TJ, Pan SF, Shinozuka H: Diversity of the promoting action of cyclosporine on the induction of murine lymphoid tumors. Carcinogenesis 9:1091, 1988.
18. Dummer JS, Bound LM, Singh G, Atchison RW, Kapadia SB, Ho M: Epstein-Barr virus-induced lymphoma in a cardiac transplant recipient. Am J Med 77:179, 1984.
19. Bennett WM, Norman DJ: Action and toxicity of cyclosporine. Ann Rev Med 37:215, 1986.
20. Walz G, Zanker B, Melton LB, Suthanthiran M, Strom TB: Possible association of the immunosuppressive and B cell lymphoma-promoting properties of cyclosporine. Transplant 49:191, 1990.
21. Penn I, Brunson ME: Cancers after cyclosporine therapy. Transplant Proc 20:885, 1988.
22. Penn I: Cyclosporine and oncogenesis. Mt Sinai J Med (NY) 54:460, 1984.
23. Yabu K, Warty VS, Gorelik E, Shinozuka H: Cyclosporine promotes the induction of thymic lymphomas in C57BL/6 mice initiated by a single dose of gamma radiation. Carcinogenesis 12:43, 1991.
24. Luciani J, Benalia H, Bitker MO, Mouquet C, Chartier-Kastler E, Ourhama S, Jacobs C, Chatelain C, Viars P: Influence of antilymphocyte globulin upon the incidence of malignancies after kidney transplantation. Transplant Proc 25:2302, 1993.
25. Mehta AB, Chiu E, Harhalakis N, Economou K, Foroni L, Luzzalto L, Gordon-Smith EC: A T-cell lymphoma of suppressor phenotype arising in a patient with severe aplastic anemia. Br J Haematol 72: 287, 1989.
26. Kheirbek AO, Molnar ZV, Choudhury A, Geis WP, Daugirdas JT, Hano JE, Ing TS: Malignant lymphoma in a renal transplant recipient treated with antithymocyte globulin. Transplant 35:267, 1983.
27. Frizzera G, Hanto DW, Gajl-Peczalska KJ, Rosai J, McKenna RW, Sibley RK, Holahan KP, Lindquist LL: Polymorphic diffuse B-cell hyperplasias and lymphomas in renal transplant recipients. Cancer Res 41:4262, 1981.